

unmethylated cytosine residues to uracil residues. Preferably, the PCR reaction uses the following primers to amplify at least a portion of the WT1 antisense regulatory region:

Tf: 5'-GGGTGGAGAAGAAGGATATATTTAT-3'. (SEQ ID NO: 1)

Tr: 5'-TAAATATCAAATTAATTTCTCATCC-3'. (SEQ ID NO: 2)

TfN: 5'-GATATATTTATTTATTAGTTTTGGT-3' (SEQ ID NO: 3; nested primer).

TrN: 5'-AAACCCCTATAATTTACCCTCTTC-3' (SEQ ID NO: 4; nested primer).

Please replace the paragraph on page 6, lines 21-25 with the following paragraph.

The nested PCR reaction involves the following primers.

Tf: 5'-GGGTGGAGAAGAAGGATATATTTAT-3'. (SEQ ID NO: 1)

Tr: 5'-TAAATATCAAATTAATTTCTCATCC-3'. (SEQ ID NO: 2)

TfN: 5'-GATATATTTATTTATTAGTTTTGGT-3' (SEQ ID NO: 3; nested primer).

TrN: 5'-AAACCCCTATAATTTACCCTCTTC-3' (SEQ ID NO: 4; nested primer).

Please replace the paragraph on page 7, lines 16-20 with the following paragraph.

The RT-PCR may use two primers designed to anneal to a tumour-specific gene sequence on opposite sides of an allelic polymorphism which introduces a restriction-site in one allele only.

For example, in the case of WT, the RT-PCR may use the following primers:

Primer 1: WT18 [CTTAGCACTTTCTTCTTGGC] (SEQ ID NO: 5)

Primer 2: WITKBF2 [TTGCTCAGTGATTGACCAGG] (SEQ ID NO: 6)

Please replace the paragraph on page 8, lines 7-9 with the following paragraph.

Nucleotide sequences, and methods of disease diagnosis, detection and prognosis in accordance with the invention will now be described, by way of example only, with reference to

accompanying Figures 1(A) to 3(B), and SEQ ID NO: 8 to SEQ ID NO: 10 in which;

Please replace the description on page 8, lines 24-30 with the following description.

Figure 3(B) shows a southern blot of the antisense WT1 RNA RT-PCR products; and

SEQ ID NO: 8 shows a nucleotide sequence of the WT1 ARR; and

SEQ ID NO: 9 shows a nucleotide sequence of a negative regulatory element of a gene encoding WT-1; and

SEQ ID NO: 10 shows the nucleotide sequence of a WT1 antisense region (Gessler, M & Bruns (1993) Genomics 17:499-501) with the RT-PCR primers shown as arrows and the exonic sequences indicated in bold.

Please replace the paragraph beginning on page 11, line 23 and ending on page 12, line 1 with the following paragraph.

Illustrative primers which may be used for methylation-specific PCR are shown below, and their hybridization positions to the WT1 sequence are shown by arrows in Figure 2 for top-strand amplification. Allowing for C→T conversion, these are:

Tf: 5'-GGGTGGAGAAGAAGGATATATTTAT-3'. (SEQ ID NO: 1)

Tr: 5'-TAAATATCAAATTAATTTCTCATCC-3'. (SEQ ID NO: 2)

TfN: 5'-GATATATTTATTTATTAGTTTTGGT-3' (SEQ ID NO: 3; nested primer).

TrN: 5'-AAACCCCTATAATTTACCCTCTTC-3' (SEQ ID NO: 4; nested primer).

Please replace the paragraph on page 13, lines 18-25 with the following paragraph.

In order to determine whether the differential methylation of the *WT1* ARR/NRE is accompanied by allele specific expression of the WT1 antisense RNA (*WT1-AS*), reverse transcription-PCR (RT-PCR) analysis was conducted on fetal and normal kidney cells, and WT cells. Primers

either side of the antisense *WT1* RNA splice (see SEQ ID NO: 10 and Figure 3A) (Gessler, M., and Bruns (1993), *Genomics*, 17: 499-501, 1993) were used for RT-PCR:

Primer 1: WT18 [CTTAGCACTTTCTTCTTGGC] SEQ ID NO: 5

Primer 2: WITKBF2 [TTGCTCAGTGATTGACCAGG]. SEQ ID NO: 6

Please replace the paragraph on page 14, lines 7-14 with the following paragraph.

The PCR products obtained were digested by adding the restriction enzyme *MnII* directly to the PCR mix and incubating for 60 minutes at 37°C. The PCR products were then separated on 2% agarose gels and then alkali blotted onto Hybond N⁺ membrane and hybridized with a ³²P-labelled antisense cDNA probe. The sequence of the probe is shown in bold between WT18 and WITKBP2 in SEQ ID NO: 10. The following primers were used as DNA controls:

Primer 1: WITKBF2 [TTGCTCAGTGATTGACCAGG] (SEQ ID NO: 6)

Primer 2: WITKBR2 [TTGGCTGGAAAGCTTGCAGC] (SEQ ID NO: 7)

Please replace line 7 on page 15 with the following line.

SEQ ID NO: 8

Please replace line 7 on page 16 with the following line.

SEQ ID NO: 9

Please replace line 1 on page 17 with the following line.

SEQ ID NO: 10

Please replace the sequence listing in the application with the enclosed paper copy of the sequence listing.